ISSN: 2583-9365

Anti-inflammatory activity of ethanolic extract of *hemidesmus indicus* leaves in experimental animals

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Abstract

Animals are used in research to study some of cell structures and physiological and pathological processes. The results obtained are correlated to the human body. The acute toxicity study of the fractions and isolated pytoconstituents of *Hemidesmus indicus* was performed as per OECD guidelines 425 at a dose of 4000mg/kg. The present study was carried out to evaluate the anti-inflammatory activity of ethanolic extract of *Hemidesmus indicus* leaves in experimental animals using *in vivo* model like carrageenan induced rat paw oedema method and invitro method like Inhibition of albumin denaturation. In this method, the rats were tested with oral administration of ethanolic extract of *Hemidesmus indicus* leaves as a test drug.

Keywords: *Hemidesmus indicus*, Anti-inflammatory, Carrageenan, Albumin

Introduction

nflammation can be defined as "the local response of living mammalian tissues to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues.'' [1]. *Hemidesmus indicus* (L.) commonly called as Indian Sarsaparilla or Anantamul belongs to the family Apocynacea. The drugs currently in use for treatment of inflammation possess a mild to severe side effects. Hence there is a need of herbal formulation for better therapeutic effect [2].

Traditional pharmacological actions of Hemidesmus indicus (Kala et al., 2006)

Table 1: Various pharmacological actions of *Hemidesmus indicus* plant as described in herbal medication literature.

Effect	Actions
Skin diseases	It is useful in treating Pitta, Dosha, Erysipelas, Psoriasis, and urticaria from heat and eczema. It cleans the blood and reduces itching. It also has wound healing action.
Cardiac effect	It has cardioprotective effect and has significant role in treating cardiovascular disorders.
Diuretic effect	It reduces burning sensation during urination by cooling the urinary tract. It finds use in treating urinary infections, cystitis, urethritis, kidney infections and prostatitis.
Nervous disorders	It is particularly good for disturbed, angry or irritated emotions due to high pitta aggravating the equilibrium of the mind because of its sweet and cooling effect it nourishes pitta and mind. It has acetylcholinestrase inhibitory activity.
Anti-bacterial effect	Root extract of *Hemidesmus indicus* has anti- bacterial property. It also used in treating ringworm, thrush, and bacterial-related skin diseases.
Anti-cancer effect	It has potential anti-cancer effects. It is capable of inducing apoptosis as well as differentiation. It has potent anti-leukemic activity in human cell lines.
Other effects	Anti –diabetic effect, anti-diarrheal effect, anti-hyperlipidemic effect, anti-oxidant effect, anti-ulcerogenic effect, antivenom effect, hepato-protective effect, renoprotective effect.

Materials and methods

Preparation of Extract

Hemidesmus indicus leaves were sun dried, powdered grossly and macerated. The powdered leaf was macerated with ethanol and held in a closed chamber for seven days with stirring ocassionally. After seven days, the whole solvent extract was extracted and the concentrate evaporated to produce a syrupy consistency in a water bath. For further usage extract was kept in a desicator.

Preliminary qualitative phytochemical investigation [4,5,6)

To investigate the active constituents present in the ethanolic extract of *Hemidesmus indicus* leaves, the extract was subjected for qualitative phytochemical examination. The chemical tests used were as follows:

- ✓ **Flavonoids**
- ➢ **Shinoda test**

To the 2mg of extract 5ml of ethanol is added. The mixture is dissolved. To this 10 drops of hydrochloric chloric is added followed by the addition of small piece of magnessium. The resulting pink or reddish brown colour indicates the presence of flavanoids

- ✓ **Saponins**

2mg of extract is dissolved in a water and shaken vigorusly then kept aside for sometime. The formation of a honeycomb like a froth indicates the presence of a saponins.

- ✓ **Tannins**

To the 2mg of extract 1% ferric chloride solution is added. The formation of brownish green colour indicates the presence of tannins.

- ✓ **Glycosides**
- ➢ **Molisch test**

To the 2mg of extract 20% of alcholic α-napthol solution was added. To this mixture conc.sulphuric acid is added along the sides of the test tube. The resulting violet ring indicates the presence of glycosides. The excess addition of alkaline substance results in the disappear of violet rin.

Selection of animals

The animals used in the study are wistar rats of weighing 150-200g and swiss albino mice of weighing 25-30g of either sex. The animals are procured from animal house of N.G.S.M Institute of Pharmaceutical Sciences, Deralakatte, Mangaluru. The animals are maintained under a standard temperature of 22±2°c and humidity 55±5°c followed by 12h day and night cycle. They are housed in sanitized cages. They are allowed to access dry pellet diet and water ad libitum. The protocol is approved by institutional animal ethical committee (IAEC), (NGSMIPS/IAEC/MARCH-2019/137). According to the CPCSEA guidelines animal experiment was carried out.

Acute toxicity studies [7, 8]

- Preliminary pharmacological studies were conducted to assess the acute toxicity and LD_{50} of the ethanolic extract of *Hemidesmus indicus* leaves.
- The acute toxicity studies were carried out in adult male albino rats, which were weighing about 150-200 gms by "Up and Down" method (OECD guidelines 425).
- Overnight fasted animals were administered ethanolic bark extract at a dose of 2000 mg/kg body weight orally.
- The animals were observed for the next 4 hours for the general behavioral changes, neurological profiles and autonomic profiles, and finally for death after 24 hours.

Carrageenan induced rat paw edema method [9]

It is a method employed to evaluate the edema formed after administering a irritating agent such as carrageenan into the left hind paw. The drug treated animals are compared with the control group animals. The paw volume before treating an irritant and after treatment can be recorded. The intensity of inflammation is depends on the type of irritants being used..

Procedure

The rats of either sex male or female having a body weight of 150-200g are used. Overnight the animals are starved. The rats can consume water to get a proper hydration. The rats are divided into 5 groups and 6 animals in each group. They are categorised as follows:

Table 2 : Experimental design of Carrageenan induced rat paw edema method

Group	Treatment
Group I	Normal control (Normal saline) i.p
Group II	Standard drug (Diclofenac sodium, 10mg/kg) i.p
Group III	Leaf extract of *H.indicus* (100 mg/kg) p.o
Group IV	Leaf extract of *H.indicus* (200 mg/kg) p.o
Group V	Leaf extract of *H.indicus* (400 mg/kg) p.o

At the lateral malleolus the paw is marked with a marker. In subsequent reading the paw is dipped upto this mark and using plethysmograph paw volume is measured. 1% of carrageenan in normal saline is injected into the subplantar region of left hind paw 30miuntes after administration of drug substances. The volume is measured for 30min, 60min, 120min, and 180min.

Evaluation

The increase in paw edema volume after 30min, 60min, 120min and 180min are calculated as percentage inhibition and compared to the volume measured immediately after receiving a irritant. Animals that are treated effectively exhibits a less amount of edema. For each treated and control groups the difference of average values calculated for each time intervals and statistic calculation is carried out. The percentage inhibition of edema is calculated as follows:

$$\% \text{ edema inhibition} = \left(1 - \frac{Vt}{Vc}\right) X\, 100$$

Where,

V_t = In drug treated group volume of paw edema

V_c =In control group volume of paw volume

Anti-inflammatory activity assessment using in-vitro method

Inhibition of albumin denaturation [10]

The reaction combination (5 ml) would contain 0.2 ml of fresh hen's egg albumin, 2.8 ml phosphate buffer saline with pH 6.4 and 2 ml of EEHI at varying concentrations. Distilled water as a control used same amount as the test volume. Incubated at 37±2°C in a 15 minutes BOD incubator, the two mixtures were then later heated to a temperature of 70°C for around 5 minutes. When cooled, the absorbance has been measured by a blank vehicle at 660 nm (SHIMADZU, UV 3600). At concentrations of 78.125, 156.25, 312.5, 625, 1250, 2500 µg / ml, the reference drug diclofenac sodium was similarly tested, and absorption was identified..

Statistical analysis

The results were shown as a Mean ± SEM and evaluated using one-way analysis of variance (ANOVA), then Dunnette's test using the version 5 of the Graph Pad prism program. A statistically significant value of P is less than 0.05.

Results

Results of preliminary phytochemical analysis

The leaves of *Hemidesmus indicus* consists of following phytochemicals:- Cardiac glycosides, Tanins, Saponins, Coumarin olignoids like Hemidesmin-1, Hemidesmin-2, Flavonoids like Rutin, Hyperoside.

Acute toxicity studies

The ethanolic extract of *Hemidesmus indicus* leaves was found to be safe up to 2000 mg/kg body weight by oral route. After 24 hours animals were found to be well tolerated. There was no mortality and no signs of toxicity.

Carrageenan induced rat paw edema method

In a dose dependent manner orally administered EEHI leaves at doses of 200 mg/kg and 400 mg/kg significantly ($p<0.05$) inhibited the oedema formed. The result obtained is summarized as follows (Table 3).

In vitro anti-inflammatory activity by albumin denaturation method

The inhibition of protein is a main factor in inflammation activity. The ethanolic extract of *Hemidesmus indicus* was able to inhibit heat induced albumin denaturation. The extract possesses a maximum protein denaturation (Table 4, 5).

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Table 3: Effect of EEHI leaves on carrageenan induced rat paw edema method in rats

Sl. no	Treatment	Dose (mg/Kg)	30 min	60 min	120 min	180 min
1	Control	2ml/kg	0.51 ± 0.027	0.53 ± 0.023	0.54 ± 0.021	0.55 ± 0.021
2	Standard	10mg/kg	0.18± 0.018***	0.21±0.018***	0.19±0.010***	0.18±0.009***
3	Ethanolic Extract	100mg/kg	0.57 ± 0.052*	0.56 ± 0.019*	0.55 ± 0.018	0.54 ± 0.021
4	Ethanolic Extract	200mg/kg	0.42 ± 0.24**	0.42 ± 0.015**	0.42 ± 0.014**	0.41 ± 0.013**
5	Ethanolic Extract	400mg/kg	0.34 ± 0.26**	0.34 ± 0.007**	0.34 ± 0.008**	0.31± 0.015**

All values are expressed as Mean ± SEM. N=06 animals in each group.

$P<0.05$ = highly significant, $^{**}P<0.05$ =Significant

Treated groups are compared with the control group, using one-way ANOVA followed by Dunett's test.

Figure 1: Effect of EEHI leaves on carrageenan induced rat paw edema method at 30 mins.

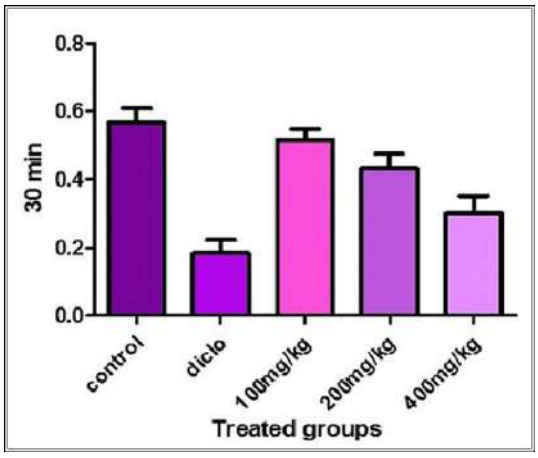

Figure 2: Effect of EEHI leaves on carrageenan induced rat paw edema method at 60 mins.

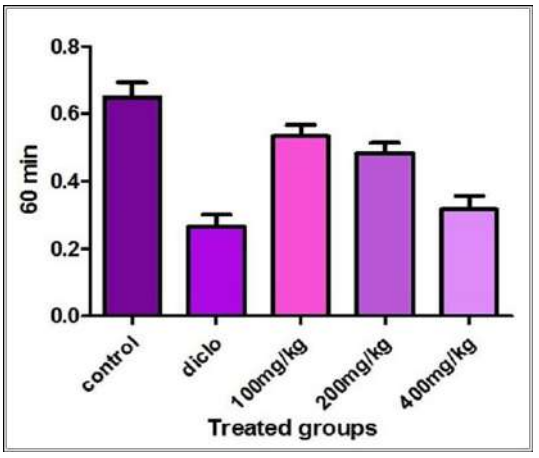

Figure 3: Effect of EEHI leaves on carrageenan induced rat paw edema method at 120 mins.

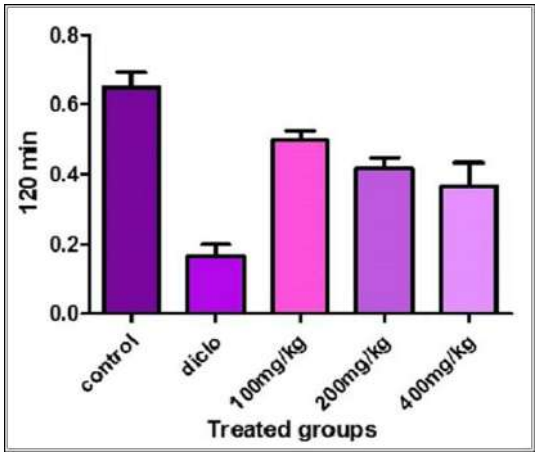

Figure 4: Effect of EEHI leaves on carrageenan induced rat paw edema method at 180 mins.

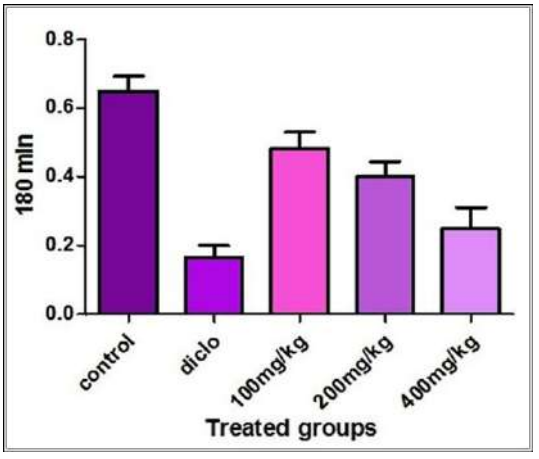

Table 4: Absorbance of ethanolic extract of *Hemidesmus indicus* leaves at 660 nm

	Concentration	Absorbance	% Inhibition
	Control	0.040	-
1	31.25	0.660	68.75
2	62.5	0.810	84
3	125	0.930	96.87
4	250	1.050	109.38
5	500	1.310	136.45
6	1000	1.390	144.79

Table 5: Absorbance of Standard (Diclofenac sodium)

	Concentration	Absorbance	% Inhibition
	Control	-	-
1	78.125	0.116	12.08
2	156.25	0.566	58.95
3	312.5	0.77	80.21
4	625	1.69	176.04
5	1250	1.76	183.33
6	2500	1.85	192.71

Discussion

The present research was undertaken to evaluate the anti-inflammatory activity of ethanolic extract of *Hemidesmus indicus* leaves in experimental animals. A preliminary phytochemical analysis stated the presence of flavonoids, saponins, tannins, coumarin olignoids. Due to the presence of various phytochemical constituents it is revealed that *Hemidesmus indicus* leaves are potential source of crude drug. Acute toxicity studies found that extract is safe upto 2000mg/kg of body weight. Anti-inflammatory activity is evaluated using in-vivo method like carageenan induced rat paw edema method and in-vitro method like inhibition of albumin denaturation. It was found that ethanolic extract of *Hemidesmus indicus* leaves exhibits a significant anti-inflammatory activity in a dose dependent manner.

Acute inflammation is evaluated by using standard experimental model such as carrageenan induced rat paw edema method. Carrageenan is used as an inducing agent and it produces a biphasic response. The initial phase i.e upto 2.5 hour after administering a inducing agent, involves a release of histamine, serotonin and kinins and in the next phase release of prostaglandin and slow reacting substance occurs which will be high at 3 hours. This results in the production of free radicals and cyclooxygenases. The cyclooxygenase inhibitors as well as lipooxygenase inhibitors are sensitive to carrageenan induced rat paw edema method which involves inhibition of prostaglandin synthesis by regulating enzyme cyclooxygenase [11]. Ethanolic extract of *Hemidesmus indicus* leaves possess a significant inhibition of rat paw edema. However the inhibition is less when compare to standard drug Diclofenac sodium. From above data it is found the 200mg/kg and 400mg/kg body weight of ethanolic extract of *Hemidesmus indicus* possess a significant inhibition of rat paw edema. From the obtained data it is correlated that the inhibition of enzyme cyclooxygenase and thereby inhibiting prostaglandin synthesis is the main mechanism through which ethanolic extract of *Hemidesmus indicus* possesses an anti-inflammatory activity [12].

To study the anti-inflammatory activity of ethanolic extract of *Hemidesmus indicus* (EEHI) leaves protein denaruration bioassay is used as an invitro method. In inflammation, denaturation of tissue protein is a main factor. The drugs which are capable to prevent protein denaturation are chosen in drug development process for inflammation. In this investigation the EEHI leaves showed increase in absorbance as compare to control. Hence EEHI has anti-inflammatory effect [13].

The results obtained in the present study revealed that the experimental animals like mice and rats treated with ethanolic leaves extract of *Hemidesmus indicus* produced a significant anti-inflammatory activity in all the animal models as well as in in-vitro study when compared to the control.

Conclusion

The present study revealed that ethanolic extract of *Hemidesmus indicus* leaves plays a major role in reliving inflammation. To understand the exact mechanism involved by the phytoconstituents present in the *Hemidesmus indicus* leaves, further studies on isolation and pytoconstituents mechanism of action need to be carried out.

Acknowledgement

The authors are thankful to authorities of NGSM Institute of Pharmaceutical Sciences, Nitte deemed to be University, Mangalore for providing all the necessary facilities.

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